REMARKS

Claims 1-49 are pending and under consideration. Claim 1 is currently amended with the insertion to the step of monitoring with the limitation, "which comprises monitoring the turning-off of the signal of the first signal probe when the temperature is below the T_m of the first quencher probe and monitoring the turning-on of the signal of the first signal probe when the temperature is above the T_m of the first quencher probe." Claim 43 is currently amended with insertion to the step of monitoring with the limitation, "which comprises monitoring the turning-off of the signals of the ***m*** and ***n*** signal probes when the temperature is below the T_m of their corresponding quencher probes and monitoring the turning-on of the signals of the ***m*** and ***n*** signal probes when the temperature is above the T_m of their corresponding quencher probes." Claims 44-49 are currently amended with insertion of the limitation "wherein each signal-quencher probe pair comprises a quencher probe having a T_m that is lower than the T_m of its corresponding signal probe" and insertion of the limitation to the step of obtaining temperature-dependent, on-off hybridization profiles for the signal-quencher probe pairs, "which comprises plotting the signal intensity during the turning-off of the signals of the signal probes when the temperature is below the T_m of their corresponding quencher probes and plotting the signal intensity during the turning-on of the signals of the signal probes when the temperature is above the T_m of their corresponding quencher probes." Support for these amendments are found throughout the specification, for example, at paragraphs [0049] and [0104] through [0110]. New claims 52-56 are new. Support for these new claims can be found, for example, at paragraphs [0068] and [0071]. No new matter is added by virtue of the amendments or the new claims. Thus, applicants respectfully request entry of this Amendment. The various rejections raised in the Office Action are discussed in more detail, below.

Rejection Under 35 U.S.C. § 102(b)

Claims 1-4, 6, 9-10, 26-28, 31-33, 36-37, 39-44, and 46-49 stand rejected under 35 U.S.C. § 102(b) as being allegedly anticipated by Wittwer et al., U.S. Patent No. 6,140,054 ("Wittwer"). Applicant traverses the rejection.

Anticipation of a claim is met "only if each and every element as set forth in the claim is found, either expressly or inherently described, in a single prior art reference." *Verdegaal Bros. v. Union Oil. Co. of California*, 814 F.2d 628, 631, 2 USPQ2d 1051, 1053 (Fed. Cir. 1987); *see also Schering Corp. v. Geneva Pharmaceuticals,* 339 F.3d 1373, 1379, 67 USPQ2d 1664, 1670 (Fed. Cir. 2003); MPEP §2131 ("The identical invention must be shown in as complete detail as is contained in the . . . claim." *Richardson v. Suzuki Motor Co.*, 868 F.2d 1226, 1236, 9 USPQ2d 1913, 1920 (Fed. Cir. 1989)). Wittwer does not disclose each and every limitation of the rejected claims.

Wittwer does not disclose a method for detecting polynucleotides by monitoring the signals from signal-quencher probe pairs as a function of temperature in the manner described by the claims. With respect to the embodiment of using signal and quencher probes that have a fluorescent resonance energy transfer relationship, the instant application states: "As one specific example, the quenching moiety may be a dye molecule capable of quenching the fluorescence of the signal fluorophores via the well-known phenomenon of FRET ... In FRET, an excited fluorophore (donor dye, in this instance the signal fluorophore) transfers its excitation energy to another chromophore (acceptor dye; in this instance the quencher)." (See Specification as filed, p. 19; underlining added.) Although the excitation energy is transferred to the acceptor dye, the specification makes clear that the invention monitors the signals from the signal fluorophore, *i.e.,* the FRET donor dye, as opposed to the conventional focus on monitoring signals from the FRET acceptor dye. In other words, the transference of excitation energy is not used to reflect a positive signal that a target sequence is present in a sample, but rather to silence or quench false-positive signals.

This difference reflects an important difference between the claims and Wittwer. When the invention uses FRET fluorophores, the signal from the acceptor fluorophore does not indicate the affirmative presence of the target polynucleotide in a sample. Rather, the acceptor fluorophore is used as a quencher, to decrease or turn-off the signal from the donor fluorophore. In this manner, the invention provides a method for detecting target sequences with higher specificity because the claims require the T_m of the signal probe to be higher than the T_m of its

corresponding quencher probe. For example, at a temperature below the Tm of the signal probe and the quencher probe, the signal probe might be able to hybridize to mismatched sequences, but the quencher probe turns-off the signal from the signal probe. When the temperature is below the Tm of the signal probe but higher than the Tm of the quencher probe, then the signal from the signal probe turns-on. By designing the quencher probe to hybridize to non-discriminating sequences in/near the target sequence, and by designing the quencher probe to have a lower T_m than the signal probe, it can be used to heighten the specificity of signal from the signal probe. Thus, by virtue of the specified relative T_ms, as the temperature is increased or decreased through a temperature range including the T_ms of the various probes, the signals produced by the signal probes turn-on or turn-off as their corresponding quencher probes either melt-off or hybridize to the target sequences. To clarify this function of temperature with respect to the monitoring of the signal-quencher probe pairs, the claims have been currently amended.

Wittwer monitors signals from a fluorescent resonance energy transfer (FRET) acceptor molecule and the present claims monitor signals from a FRET donor (where the invention involves the embodiment using FRET fluorophores). Although Figure 11 of Wittwer shows a graph of the relative fluorescence of an acceptor fluorophore (Cy5) with respect to the donor fluorophore (fluoroscein), this graph does not show the monitoring of signals from fluoroscein as a function of temperature. The claims require the monitoring of the turning-on of the signal probe (here, the fluoroscein signal or donor fluorophore) when the temperature is below the T_m of the signal probe but above the T_m of the quencher probe (here, the Cy5 signal, or the acceptor fluorophore), and the monitoring of the turning-off of the signal probe when the temperature is below the T_m of the signal and quencher probes. For Wittwer, the use of relative melting temperatures is to distinguish signals between multiple sets of FRET pairs (col. 15, ll. 6-13), and not to distinguish the turning-on or turning-off of signal within a single pair like the claimed invention.

Because the claims specify a lower Tm for the quencher probe as compared to the signal probe, monitoring the signals from the signal probe as a function of temperature provides a distinct signal profile as compared to Wittwer. For example, calculating and graphing the first

derivative of the signal profile results in a decrease or valley when the detectable signal is turned-on and an increase or peak when the detectable signal is turned-off. Over a temperature range, the profile has a peak-valley-peak or valley-peak-valley signature. (See page 31, [0107]; Fig. 2C; and Examples 1-2; Figs. 5A-5B). But with Wittwer, when the temperature increases over the T_m of the acceptor probe, then a decrease in signal results because Wittwer detects signals from acceptor probe. Thus, as compared to the claims, Wittwer provides a contrary teaching because there is a decrease of fluorescence of the acceptor fluorophore of a FRET pair in response to an increase in temperature. For example, see Wittwer at Figure 13A and Figure 17 upper panel, which depict fluorescence of various hybridized probes in relation to hybridization temperature, but which fail to show a clear fluorescence peak around the T_m upon increase of the temperature. In fact, Figure 13A and Figure 17 top panel show a continuous decrease in fluorescence as temperature increases. This signal profile is opposite to the claimed invention because Wittwer focuses on signals obtained from a FRET acceptor. Simply put, the melting temperatures of the Wittwer probes are not designed with the strategy of using the acceptor probe to quench potentially false positive signals from the donor probe.

Because Wittwer does not teach monitoring the signals from a FRET donor as a function of temperature with respect to the relative T_ms of the donor and acceptor probe pair, Wittwer does not disclose all of the limitations of the claims. For example, Wittwer does not disclose the claim 1 limitation "monitoring the turning-off of the signal of the first signal probe when the temperature is below the T_m of the first quencher probe and monitoring the turning-on of the signal of the first signal probe when the temperature is above the T_m of the first quencher probe." Wittwer does not disclose the claim 43 limitation, "monitoring the turning-off of the signals of the ***m*** and ***n*** signal probes when the temperature is below the T_m of their corresponding quencher probes and monitoring the turning-on of the signals of the ***m*** and ***n*** signal probes when the temperature is above the T_m of their corresponding quencher probes." Nor does Wittwer disclose the claim 44-49 limitation "plotting the signal intensity during the turning-off of the signals of the signal probes when the temperature is below the T_m of their corresponding quencher probes and plotting the signal intensity during the turning-on of the signals of the signal probes when the

temperature is above the T_m of their corresponding quencher probes." Because claims 2-4, 6, 9-10, 26-28, 31-33, 36-37, 39-43, and 52-56 depend from claim 1, Wittwer does not disclose all of the limitations of these claims. Applicant respectfully requests reconsideration and withdrawal of this ground of rejection.

Rejections Under 35 U.S.C. § 103(a)

A determination of obviousness is a question of law based on these factual inquiries: (1) determining the scope and content of the prior art; (2) ascertaining the differences between the prior art and the claims at issue; (3) resolving the level of ordinary skill in the pertinent art; and (4) assessing objective evidence relative to secondary indicia of nonobviousness, such as commercial success, long felt but unresolved needs, and failure of others. *In re Kahn*, 441 F.3d 977, 985, 78 USPQ2d 1329, 1334-1335 (Fed. Cir. 2006); *citing Graham v. John Deere Company of Kansas City*, 383 U.S. 1, 17-18, 86 S.Ct. 684, 148 USPQ 459, 466-467 (1966). To establish a prima facie case of obviousness, three basic criteria must be met: (1) there must be some suggestion to those of ordinary skill in the art to modify the reference or combine the reference teachings; (2) those of ordinary skill must have a reasonable expectation of success; and (3) the combination of prior art references must teach or suggest all of the claim limitations. *See Velander v. Garner*, 348 F.3d 1359, 1363, 68 USPQ2d 1769, 1772 (Fed. Cir. 2003); MPEP §2142. .

1. Wittwer and Tsourkas or Wittwer and Sokol

Claims 11-15 stand rejected under 35 U.S.C. § 103(a) as being allegedly obvious over Wittwer et in view of Tsourkas et al., 2002, *Nucleic Acids Res.* 30:5168 ("Tsourkas") or in view of Sokol et al., 1998, *Proc. Natl. Acad. Sci. USA* 95:11538-11543 ("Sokol"). Applicant traverses the rejections.

The combination of Wittwer and Tsourkas or Wittwer and Sokol does not teach or suggest all the limitations of the claims. For example, Wittwer does not disclose the claim 1 limitation "monitoring the turning-off of the signal of the first signal probe when the temperature

is below the T_m of the first quencher probe and monitoring the turning-on of the signal of the first signal probe when the temperature is above the T_m of the first quencher probe." Wittwer does not disclose the claim 43 limitation, "monitoring the turning-off of the signals of the ***m*** and ***n*** signal probes when the temperature is below the T_m of their corresponding quencher probes and monitoring the turning-on of the signals of the ***m*** and ***n*** signal probes when the temperature is above the T_m of their corresponding quencher probes." Nor does Wittwer disclose the claim 44-49 limitation "plotting the signal intensity during the turning-off of the signals of the signal probes when the temperature is below the T_m of their corresponding quencher probes and plotting the signal intensity during the turning-on of the signals of the signal probes when the temperature is above the T_m of their corresponding quencher probes."

In fact, Wittwer teaches away from monitoring the detectable signals of the signal probes as a function of temperature. As discussed above, Wittwer teaches the monitoring of the decrease of fluorescence of the acceptor fluorophore of a FRET pair in response to an increase in temperature. In contrast, the specification provides an illustrative embodiment, where "[i]n FRET, an excited fluorophore (donor dye, in this instance the signal fluorophore) transfers its excitation energy to another chromophore (acceptor dye; in this instance the quencher)." (See Specification as filed, p. 19; underlining added.) Because in the relevant embodiment the signal fluorophore in the present application is the donor molecule (as opposed to acceptor molecule in Wittwer), and because the claims specify that the quencher probe has a lower T_m than the T_m of its corresponding signal probe, the invention and Wittwer will provide distinct signal profiles as a function of temperature. Wittwer provides no teaching or suggestion to monitor the detectable signals from the signal probes as a function of temperature.

Tsourkas does not rectify this deficiency. Tsourkas is directed to the use of 2'-deoxy molecular beacons, but makes no teaching or suggestion to monitor detectable signals from such probes as a function of temperature with respect to its T_m and the T_m of corresponding quenching probes. Sokol is also directed to molecular beacons, but also lacks any teaching or suggestion to monitor detectable signals as a function of temperature. Neither Tsourkas or Sokol describes a method that can provide a signal profile where signals from a signal probe increase when the

temperature rises above the T_m of its corresponding quenching probe. Because claims 11-15 depend from claim 1, the combinations of Wittwer and Tsourkas and Wittwer and Sokol do not teach or suggest all of the limitations of claims 11-15. Applicant respectfully requests reconsideration and withdrawal of these grounds of rejection.

2. Wittwer and Kubista

Claims 11, 16-20, and 38 stand rejected under 35 U.S.C. § 103(a) as being allegedly obvious over Wittwer in view of Kubista et al., U.S. Patent No. 6,329,144 ("Kubista"). Applicant traverses the rejection.

The combination of Wittwer and Kubista does not teach or suggest all the limitations of the claims. As discussed *supra*, Wittwer does not teach or suggest at least the claim limitations that relate to monitoring signals from signal probes as a function of temperature. Kubista does not rectify this deficiency. Kubista is directed to probes having a sequence recognizing element and a reporter group joined by a linker. But Kubista makes no mention or suggestion to monitor the signals from such probes as a function of temperature with respect to corresponding quenching probes. Because claims 11, 16-20, and 38 depend from claim 1, the combination of Wittwer and Kubista does not teach or suggest all of the limitations of these claims. Applicant respectfully requests reconsideration and withdrawal of this ground of rejection.

3. Wittwer and Tyagi

Claims 11 and 21-23 stand rejected under 35 U.S.C. § 103(a) as being allegedly obvious over Wittwer in view of Tyagi et al., U.S. Patent No. 6,277,607 ("Tyagi"). Applicant traverses the rejection.

The combination of Wittwer and Tyagi does not teach or suggest all the limitations of the claims. As discussed *supra*, Wittwer does not teach or suggest at least the claim limitations that relate to monitoring signals from signal probes as a function of temperature, but rather teaches away from the limitations. Tyagi does not rectify this deficiency. Tyagi is directed to

oligonucleotide primers, including hairpin primers labeled with fluorescent moieties. But Tyagi makes no mention or suggestion to monitor the signals from such probes as a function of temperature with respect to corresponding quenching probes. Because claims 11 and 21-23 depend from claim 1, the combination of Wittwer and Tyagi does not teach or suggest all of the limitations of these claims. Applicant respectfully requests reconsideration and withdrawal of this ground of rejection.

4. Wittwer and Singer

Claims 24-25 stand rejected under 35 U.S.C. § 103(a) as being allegedly obvious over Wittwer in view of Singer et al., U.S. Patent No. 6,323,337 ("Singer"). Applicant traverses the rejection.

The combination of Wittwer and Tyagi does not teach or suggest all the limitations of the claims. As discussed *supra*, Wittwer does not teach or suggest at least the claim limitations that relate to monitoring signals from signal probes as a function of temperature, but rather teaches away from the limitations. Singer does not rectify this deficiency. Singer is directed to dye-labeled oligonucleotides that can quench the luminescence of non-covalently bound nucleic acid stains. But Tyagi makes no mention or suggestion to use such oligonucleotides as quenching probes corresponding to signal probes in methods that monitor the signals from the signal probes as a function of temperature. Because claims 24-25 depend from claim 1, the combination of Wittwer and Singer does not teach or suggest all of the limitations of these claims. Applicant respectfully requests reconsideration and withdrawal of this ground of rejection.

5. Wittwer and Schalasta

Claim 45 stands rejected under 35 U.S.C. § 103(a) as being allegedly obvious over Wittwer in view of Schalasta et al. (*Infection* 2000, Vol. 28, p. 85) ("Schalasta"). Applicant traverses the rejection.

The combination of Wittwer and Schalasta does not teach or suggest all the limitations of the claims. As discussed *supra*, Wittwer does not teach or suggest at least the claim limitations that relate to monitoring signals from signal probes as a function of temperature, but rather teaches away from the limitations. Schalasta does not rectify this deficiency. Schalasta is directed to Herpes Simplex Virus (HSV) genotyping by PCR and fluorescence melting curve analysis. But Schalasta makes no mention or suggestion to use sets of signal and quenching probes directed to HSV target sequences, where signal profiles are monitored as a function of temperature with respect to the T_ms of the signal and quenching probes as required by the claims. Applicant respectfully requests reconsideration and withdrawal of this ground of rejection.

6. Wittwer and Caplin

Claims 5 and 7-8 stand rejected under 35 U.S.C. § 103(a) as being allegedly obvious over Wittwer in view of Caplin et al., (*Biochemical*, 1999, No. 1, p. 5) ("Caplin"). Applicant traverses the rejection.

The combination of Wittwer and Caplin does not teach or suggest all the limitations of the claims. As discussed *supra*, Wittwer does not teach or suggest at least the claim limitations that relate to monitoring signals from signal probes as a function of temperature, but rather teaches away from the limitations. Caplin does not rectify this deficiency. Caplin is directed to fluorescent hybridization analysis using PCR. But Caplin makes no mention or suggestion to monitor the signals from such probes as a function of temperature with respect to corresponding quenching probes. In fact, Caplin also teaches away from the invention because it states that "[f]luorescence from the acceptor probe will only occur when both the donor probe and the acceptor probe have annealed to the product." (See Caplin at p. 5, rt. col., 1st paragraph.) Caplin does not teach or suggest that an increase or turning-on of signal from a signal probe when the temperature rises above the T_m of a corresponding quencher probe. Because claims 5 and 7-8 depend from claim 1, the combination of Wittwer and Caplin does not teach or suggest all of the limitations of these claims. Applicant respectfully requests reconsideration and withdrawal of this ground of rejection.

7. Wittwer and Marras

Claims 29 and 34 stand rejected under 35 U.S.C. § 103(a) as being allegedly obvious over Wittwer in view of Marras et al., *Nucleic Acids Research*, 2002, Vol. 30, p. e122 ("Marras"). Applicant traverses the rejection.

The combination of Wittwer and Marras does not teach or suggest all the limitations of the claims. As discussed *supra*, Wittwer does not teach or suggest at least the claim limitations that relate to monitoring signals from signal probes as a function of temperature, but rather teaches away from the limitations. Marras does not rectify this deficiency. Marras is directed to fluorescence resonance energy transfer and contact-mediated quenching in oligonucleotide probes. But Marras makes no mention or suggestion to monitor the signals from signal probes as a function of temperature with respect to corresponding quenching probes. For example, Marras provides no showing that a signal profile can be obtained where signals from a signal probe increase when the temperature rises above the T_m of its corresponding quenching probe yet is below the T_m of the signal probe. Because claims 29 and 34 depend from claim 1, the combination of Wittwer and Marras does not teach or suggest all of the limitations of these claims. Applicant respectfully requests reconsideration and withdrawal of this ground of rejection.

8. Wittwer and Elenitoba-Johnson

Claims 29 and 34 stand rejected under 35 U.S.C. § 103(a) as being allegedly obvious over Wittwer in view of Elenitoba-Johnson, U.S. Patent No. 6,346,386 ("Elenitoba-Johnson"). Applicant traverses the rejection.

The combination of Wittwer and Elenitoba-Johnson does not teach or suggest all the limitations of the claims. As discussed *supra*, Wittwer does not teach or suggest at least the claim limitations that relate to monitoring signals from signal probes as a function of temperature, but rather teaches away from the limitations. Elenitoba-Johnson does not rectify this deficiency. Elenitoba-Johnson is directed to a method for determining whether a DNA

sample is identical to a wild-type sequence, where the method is purportedly sensitive enough to detect a change of a single base pair. Elenitoba-Johnson uses fluorescent labels to monitor the difference in melting temperatures between a primer with a high GC content and a target sequence. But Elenitoba-Johnson makes no mention or suggestion to monitor the signals from a signal probe as a function of temperature with respect to the T_m of the signal and quencher probes. For example, Elenitoba-Johnson does not provide a signal profile that exhibits an increase in signal from a signal probe when the temperature is below the T_m of the signal probe and above the T_m of a corresponding quencher probe. Because claims 29 and 34 depend from claim 1, the combination of Wittwer and Elenitoba-Johnson does not teach or suggest all of the limitations of these claims. Applicant respectfully requests reconsideration and withdrawal of this ground of rejection.

9. Wittwer and Wittwer B

Claims 30 and 35 stand rejected under 35 U.S.C. § 103(a) as being allegedly obvious over Wittwer in view of Wittwer, U.S. Patent No. 6,245,514 ("Wittwer B"). Applicant traverses the rejection.

The combination of Wittwer and Wittwer B does not teach or suggest all the limitations of the claims. As discussed *supra*, Wittwer does not teach or suggest at least the claim limitations that relate to monitoring signals from signal probes as a function of temperature, but rather teaches away from the limitations. Wittwer B does not rectify this deficiency. Wittwer B is directed to FRET pairs for detecting the presence of a target analyte where the donor fluorophore's emission spectrum and the acceptor fluorophore's absorption spectrum overlap by less than 25% (see Wittwer B, Abstract). Wittwer B makes no mention or suggestion to monitor signals from signal probes as a function of temperature in the manner of the claims. Like Wittwer, the signals that are detected in Wittwer B are from an acceptor fluorophore. As discussed above, in an illustrative embodiment of the invention, the signal probe is a FRET donor fluorophore and the quencher probe is a FRET acceptor fluorophore. This is opposite to Wittwer and Wittwer B. Because of this inverse relationship, neither Wittwer nor Wittwer B can

obtain a signal profile from a signal probe as a function of temperature where signals increase or turn-on when the temperature is below the T_m of the signal probe and above the T_m of the quencher probe. Rather, Wittwer B shows that fluorescence signals decrease as a continuum with respect to increasing temperatures (see, for example, Figures 13, 31-41 in Wittwer B.) Because claims 30 and 35 depend from claim 1, the combination of Wittwer and Wittwer B does not teach or suggest all of the limitations of these claims. Applicant respectfully requests reconsideration and withdrawal of this ground of rejection.

Conclusion

Claims 1-49 and 52-56 are believed to satisfy all of the criteria for patentability and are in condition for allowance. An early indication of the same is therefore kindly requested.

No fees beyond those for the Petition for an Extension of Time that accompanies this Amendment are believed to be due. However, the Commissioner is authorized to charge any additional fees that may required, or credit any overpayment, to Dechert LLP Deposit Account No. 50-2778 (**Order No. 375461-036US (355414)**).

Respectfully submitted,

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